itraconazole and terbinafine against clinical isolates of *Scedosporium prolificans* Results from animal experiments are still scarce and difficult to interpret because of altered pharmacokinetics in most animals. In humans, synergism seems to be present for amphotericine combined with flucytosine to treat infections caused by *Cryptococcus spp.* It is expected, that new methods to determine synergism between antifungals will result in a more ratinoal approach of using combinations of antimycotics in humans.

S13 – New strategies for treatment of viral infections

TuS9 T cell therapy of HCMV infection

Hermann Einsele
Medizinische Klinik II, Eberhard-Karls-University, Tübingen, Germany

The reconstitution of HCMV-specific immune responses after allogeneic SCT has been demonstrated to be protective against the development of HCMV disease. S. Riddell and P. Greenberg have shown protective HCMV-specific T cell immunity to be transfered to the recipients of an allogeneic stem cell transplant by the infusion of donor-derived ex vivo generated HCMV-specific cytotoxic T cell (CTL) clones. Alternative strategies to deplete of alloreactive T cells and/or enrich for CMV specific T cells in donor PBMNCs are increasingly explored and will be discussed. One possibility is to pulse dendritic cells, the "professional" antigen presenting cells, with soluble synthetic peptides to induce and propagate HCMV specific CTLs from HCMV-seropositive and also HCMV-seronegative donors. After repetitive specific stimulation T cell lines highly enriched for HCMV specific T cells can be generated and safely transferred to patients. In a phase I/II-study recipients of an allograft with persistent HCMV infection in spite of prolonged antiviral chemotherapy received $1 \times 10^7/m^2$ polyclonal cell lines without any significant side effects. A transient reduction of viral load could be documented in all these patients, the majority of them showed sucessful control of HCMV infection.

TuS11 Ribozyme and stem cell gene therapy for the treatment of HIV infection

J. Rossi[1], D. Castanotto[1], H. Li[1], A. Michienzi[1], A. Krishna[4], D. Kohn[2], G. Bauer[2], J. M. Rossi[1], J. A. Zaia[3]
[1]*Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA;* [2] *Childrens' Hospital of Los Angeles, USC School of Medicine;* [3]*Department of Virology, Beckman Research Institute of the City of Hope, Duarte, CA;* [4]*Division of Hematology and Bone Marrow Transplantation, City of Hope National Medical Center, Duarte, CA, United States*

Objectives: Genetic modification of hematopoietic stem cells with anti-HIV catalytic RNAs (Ribozymes).
Methods: Hammerhead rihozymes targeting the HIV-1 *tat* and *rev* transcripts are transduced in the backbone of a murine retroviral vector into primary hetamotpoietic progenitor cells from HIV-1 infected individuals. The ribozymes are expressed as a polycistronic transcript from the LTR of the LN retroviral vector. Our hypothesis is that stable transduction of pluripotent hematopoietic stem cells by ribozyme expressing vectors followed by engraftment of these cells in the marrow, will ultimately provide a population of HIV-1 resistant T-cells, monocytes, and dendritic cells. To test this, we have initiated a clinical trial involving HIV-1 infected individuals who have AIDS related lymphoma using vectors harboring ribozyme or vector backbone alone transduced into their stem cells. In parallel to the clinical studies, we are testing new strategies for ribozyme-target co-localization using chimeric RNA molecules, which harbor our rihozymes of interest tethered to sequences, which direct the ribozymes to discrete intracellular localizations.
Results: The clinical trial studies have demonstrated a selective survival of ribozyme expressing cells within a period of several weeks following bone marrow transplantation and long term engraftment in at least one patient. Our new chimeric ribozyme localization studies demonstrate that a nucleolar-localized ribozyme is a potent inhibitor of HIV-1.
Conclusions: Ribozyme mediated gene therapy for the treatment of HIV-1 infection is feasible utilizing hematopoietic stem cells. Potent inhibition of HIV-1 infection by a nucleolar ribozyme suggests nucleolar trafficking of HIV-1 RNAs, opening new targets for anti-HIV-1 therapies.

S14 – Assesment of respons to antiviral therapy...

TuS13 Assessment of response to antiviral therapy and antiviral resistance: Hepatitis B and C virus

David Gretch
University of Washington, Laboratory Medicine, Settle, Washington 98104, United States

Chronic viral hepatitis due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is a major cause of morbidity and mortality, effecting hundreds of millions of individuals worldwide. Long term complications of untreated chronic viral hepatitis include progressive liver disease and hepatocellular carcinoma. Interferon alpha has historically been the first line treatment for chronic viral hepatitis. However, this therapy is only moderately effective and is often limited by side effects. Newer agents including nucleoside analogs for chronic hepatitis B, and ribavirin in combination with interferon for chronic hepatitis C, have shown promise for improving response rates. In chronic hepatitis B, antigen tests (HBeAg, HBsAg), antibody tests (anti-HBs, anti-HBe) and nucleic acid tests (HBV DNA) are used to monitor response to antiviral therapy, while in chronic hepatitis C, treatment response is monitored by following qualitative and/or quantitative HCV RNA in serum. In general, successful treatment of chronic hepatitis B results in disappearance of the viral antigens and HBV DNA, and seroconversion to positive anti-HBs and anti-HBe status. In chronic hepatitis C, successful treatment is associated with clearance of HCV RNA from serum during the first 3–6 months of therapy, and absence of detectable viral RNA in serum 6 months after cessation of therapy. In both cases, such responses predict sustained clinical remissions and histological improvement of liver disease. Drug resistance to nucleoside analog monotherapy is well documented in chronic hepatitis B, and appears to result from critical mutations within the HBV polymerase gene. The role of viral mutations in determining sensitivity or resistance of HBV and HCV to interferon therapy is less well defined, primarily due to a lack of suitable culture systems for phenotypic characterization of putative resistant viral populations.

TuS16 Detection of drug-resistant HCMV strains by monitoring response to antiviral treatment in immunocompromised patients

G. Gerna, F. Baldanti, D. Lilleri, A. Sarasini
Servizio di Virologia, IRCCS Policlinico S. Matteo, Pavia, Italy

Monitoring of response to treatment by quantitative determination of antigenemia viremia and leukoDNAemia is the most useful tool for early detection of drug-resistant HCMV strains in immunocompromised patients. In fact, lack of reduction or increase in level of measured viral parameters in blood during therapy suggests the unrestricted viral replication. In particular, while antigenemia and leukoDNAemia quantitate viral components, viremia is a direct measure of the amount of infectious virus and of the virus replicative potential during treatment. However, delayed reduction in viral load during treatment is not invariably associated with the emergence of a drug-resistant HCMV strain. Thus, rapid confirmatory assays are needed in order to decide treatment change. A simplified immediate- early plaque-reduction assay using blood leukocytes as inoculum, allows a rapid (4–6 days) screening for drug-resistance. Detection of specific mutations in HCMV UL97 or UL54 genes directly in clinical specimens by PCR-based methods or sequencing allows demonstration of the presence of drug-resistant strains within 2–3 days. In AIDS patients, disseminated HCMV infections have been treated mostly in the presence of specific symptoms, whereas in solid organs or bone marrow recipients adoption of preemptive therapy protocols implies initiation of anti-HCMV treatment when patients are still in the asymptomatic phase of the infection. This difference along with the need in the recent past of prolonged anti-HCMV maintenance treatment in AIDS patients might account for the reported greater prevalence of HCMV drug-resistant strains in this patient population with respect to transplant recipients. However, the recent introduction of potent antiretroviral combination protocols was followed by a drastic reduction in the prevalence of HCMV infections in AIDS patients, with a further reduced number of reported new resistant HCMV strains. In the transplantation settings, several anedoctical reports of HCMV drug-resistant strains points to a possible emerging problem in the near future.